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SIMPLE SEQUENCE REPEAT DNA MARKERS LINKED WITH GENES FOR RESISTANCE TO MAJOR DISEASES OF COMMON BEAN

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INTRODUCTION: Rust, anthracnose (ANT), and angular leaf spot (ALS) are widespread and devastating diseases of the common bean in Latin America and Africa. Using cultivars with disease resistance is the most cost-effective strategy to manage these diseases. Single and dominant genes condition the resistance to rust, ANT, and ALS in common bean. Combining different genes into single cultivars to manage one or more of these diseases is one of the main objectives of many common bean breeding programs. Bean cultivars with broad resistance to some of these diseases have been developed by combining several resistance genes using traditional, laborious, and time-consuming methods to pyramid these genes. To accelerate and facilitate gene pyramiding, effective, inexpensive, and easy to use DNA markers are needed. Simple sequence repeat (SSR) DNA markers are effective, co-dominant, and easy to visualize in most laboratories in developing or developed nations. The objective of this study was to discover SSR markers closely linked to genes for resistance to the rust, ANT, and ALS diseases.

MATERIAL AND METHODS: F₂ populations for the rust resistance genes reported here were developed using the following crosses: Pinto 114 × Aurora (*Ur-3*), Mexico 309 (*Ur-5*) × Early Gallatin (*Ur-4*), Pinto 114 × PI 181996 (*Ur-11*), Pinto 114 × PI 310762 (*Ur-PI₃₁₀₇₆₂*). The parents and F₂ segregating populations were evaluated for their reaction to specific races of bean rust pathogen. The *Ur-14* rust resistance gene and the gene cluster *Co-3⁴/Phg-3* that confer resistance to anthracnose (*Co-3⁴*) and ALS (*Phg-3*) present in Ouro Negro cultivar were studied using 105 F_{2:3} families from the Rudá × Ouro Negro cross. Co-segregation analysis between *Ur-14* gene and the *Co-3⁴/Phg-3* loci was performed using these F_{2:3} families, which were evaluated for their reaction to the rust and anthracnose pathogens independently. Total genomic DNA was isolated from the parents and the segregating population. To perform the molecular analysis, susceptible F₂ plants were used for bulk segregant analysis (BSA) for *Ur-3*, *Ur-4*, *Ur-5*, *Ur-11* and *Ur-PI₃₁₀₇₆₂*. Genotyping was also performed on the 105 F_{2:3} families from the Rudá × Ouro cross. The DNA from the parents, the susceptible bulks and the 105 F_{2:3} families were screened using the BARCBear6K_3 Illumina BeadChip with 5,399 single nucleotide polymorphism (SNP) DNA markers following the Infinium assay. SNP alleles were called using the Genome Studio and all allele data were manually checked. Positive hits for BSA were recorded when a SNP was polymorphic between the susceptible and the resistant parents and susceptible bulks clustered with the susceptible parent. Similarly, positive markers for *Ur-14* and *Co-3⁴/Phg-3* were recorded when a SNP was polymorphic between Rudá and Ouro Negro. Susceptible F_{2:3} families clustered tightly with Rudá (S), resistant F_{2:3} families clustered tightly with Ouro Negro (R), and the heterozygous families were in a separate group. The sequence scaffolds containing polymorphic SNPs were evaluated for the presence of SSR using the Perl script “MISA”. Flanking markers to the SSR regions were designed using Primer3. Each primer pair was used to amplify genomic DNA of the parents. Polymorphic SSR markers were validated in the segregating populations. Genomic DNA of each plant was amplified using PCR and the products were then analyzed using agarose gel electrophoresis.

RESULTS: We have used existing new technologies for SNP genotyping to identify SSR DNA markers closely linked with genes that confer resistance to the rust, ANT, and ALS diseases of common bean. BSA and the BARCBean6K_3 chip were used to identify many SSR DNA markers linked to several important genes that confer resistance to the pathogens that cause rust (*Ur-3*, *Ur-4*, *Ur-5*, *Ur-11*, and *Ur-PI₃₁₀₇₆₂*) in common bean. We have identified three flanking SSR markers linked to *Ur-4* that were very effective in identifying Andean and Mesoamerican beans with the *Ur-4* gene alone or in combination with other rust resistance genes. This is a significant improvement over a previously published molecular marker linked to *Ur-4* (OA14₁₁₀₀) that was present in all Andean bean lines with or without the *Ur-4* gene. Furthermore, we have also identified the SSR markers BARCPVSSR14078 and BARCPVSSR04582, linked to *Ur-5* at 0.0 cM. These markers were effective in differentiating cultivars with *Ur-5* from those without this gene. We also identified two SSR markers linked to *Ur-14* at 0.0 cM. The close physical linkage between the *Ur-14* and the *Co-3⁴/Phg-3* cluster ensures that these genes are inherited together. Thus, the SSR markers discovered during this study are most useful to detect the presence of the three genes in this cluster with resistance to rust, ANT, and ALS. Moreover, some of the SSR markers discovered in this study are flanking and closely linked to genes conditioning resistance to these three diseases of common bean. These markers would be very effective when used in marker-assisted selection. We still searching for new, specific, thus more effective, SSR markers linked to *Ur-3* and *Ur-11*.

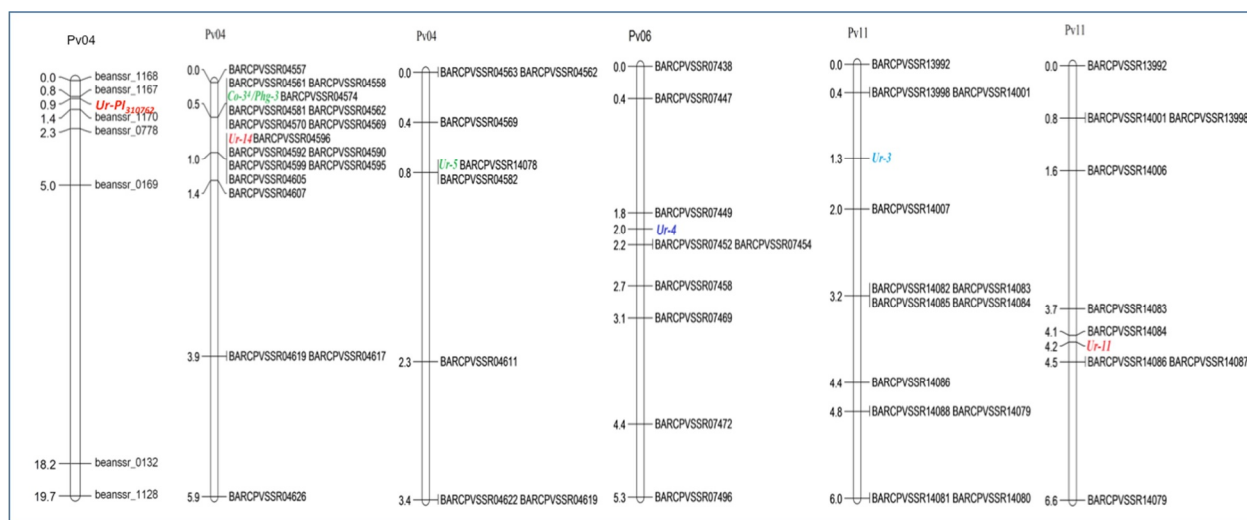


Figure 1. Genetic linkage maps of the major resistance genes: *Ur-14*, *Co-3⁴/Phg-3* ($F_{2,3}$ Rudá \times Ouro Negro); *Ur-PI₃₁₀₇₆₂* (F_2 Pinto 114 \times PI 310762); *Ur-5*, *Ur-4* (F_2 Mexico 309 \times Early Gallatin); *Ur-3* (F_2 Pinto 114 \times Aurora); *Ur-11* (F_2 Pinto 114 \times PI 181996).